BioNutrients-2 (BN-2) Payload Overview

POIWG #50

April 19-21, 2022

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Ames Research Center (ARC)


Thank you
NASA
SILICON VALLEY
AMES RESEARCH CENTER

BioNutrients-2 Experiment Summary

Principal Investigators	John Hogan, Ph.D. NASA Ames Research Center
Sponsor	Technology and Science Research Office
Funding Authority	NASA / Human Exploration & Operations / Space Technology Mission Directorate (STMD)
Experiment Duration	Approximately 6 months from launch
Ground Control	Near-synchronous Ground Control performed at PI Laboratory at Ames
Research Objectives	The goal of the BioNutrients experiment is to determine the effect of long-duration, low-Earth-orbit stowage on the ability to biologically generate nutrients through organism activation and growth.

BioNutrients Key Stakeholders

- **PI** – The Principal Investigator of the BioNutrients Project, Dr. John Hogan at NASA Ames Research Center
- **STMD** – The Space Technology Mission Directorate – Game Changing Division – Advanced ECLSS and ISRU at NASA Headquarters
- **HRP** – The Human Research Program Office at NASA Johnson Space Center
- **ISS** – The ISS Payload Program at NASA Johnson Space Center
- **Code S** – The ISS Utilization Office in the Science Directorate at NASA Ames Research Center
- **Code SC** – The Space Biosciences Division at NASA Ames Research Center
- **Code SCF** – The Flight Systems Implementation Branch at NASA Ames Research Center
- **Code SCB** – The Bioengineering Branch at NASA Ames Research Center
- **ARC OCE** – The Office of the Chief Engineer at NASA Ames Research Center

Purpose and Goals

BioNutrients Project Purpose: To enable rapid, safe and reliable *in situ* production of needed dietary nutrients using minimal mass, power and volume for long duration missions.

Goals of the BioNutrients-2 Payload:

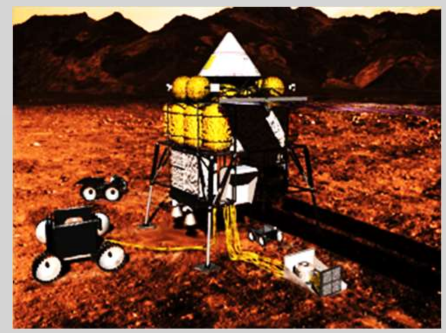
1. To enable growth of selected organisms
2. To produce targeted nutritive compounds on ISS
3. To improve mass/power/volume as compared to the BioNutrients-1 Payload
4. To expand capabilities to support additional products and/or organisms
5. To incorporate safety processes required for operational use by spaceflight crew members

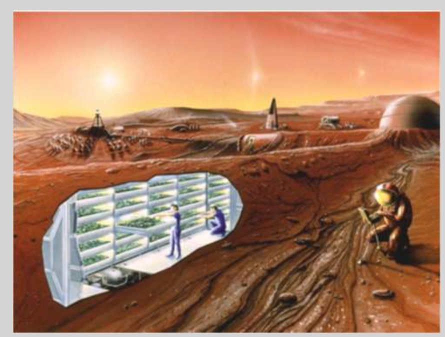
BioNutrients Background

In Space Production of Mission Products

A wide array of different mission resources will eventually need to be produced on-site for mission sustainability/cost effectiveness

- Fuels, foods/nutrients, chemicals, plastics, binders, medicine
- Biomanufacturing can provide compounds abiotic systems cannot





Increasing Capability

Future missions require new methods to provide sustainability:

- ***In situ* Resource Utilization (ISRU)** generates supplies from local resources.
- ***In situ* Manufacturing (ISM)** provides capability to make needed chemicals, fuels, building materials, pharmaceuticals, etc. on-site and on-demand.
- **Closed-loop life support systems** treat and recover valuable resources via regenerative air, wastewater, and solid waste processing systems.
- **Space medicine** systems requires the ability to monitor and maintain crew health under very adverse conditions.
- **Food production systems** are needed to supply certain nutritional needs not met by current food provisioning systems.
- These systems require increased **reliability and self-sustainability,** and decreased mass, power, volume, and consumable use.

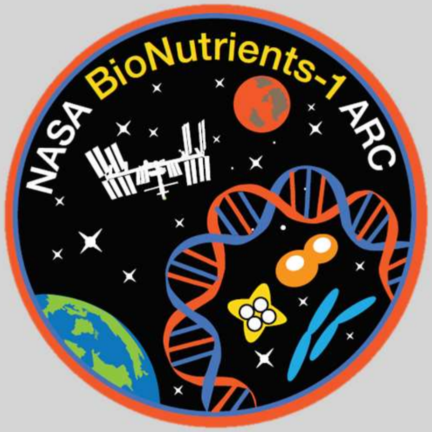
Image Credits: NASA

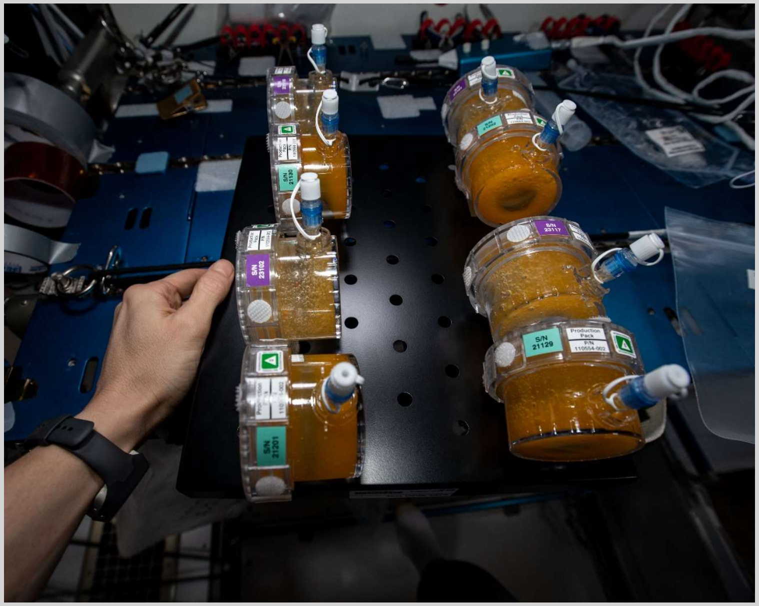
Nutrition in Space

- Enhanced nutritional needs in space travel
- Radiation and microgravity countermeasures
- Disease-specific concerns
- Psychology of food/isolation
- Nutrients degrade with time in food and supplements
- **Need to produce some foods *in situ***

BioNutrients-1 (BN-1)

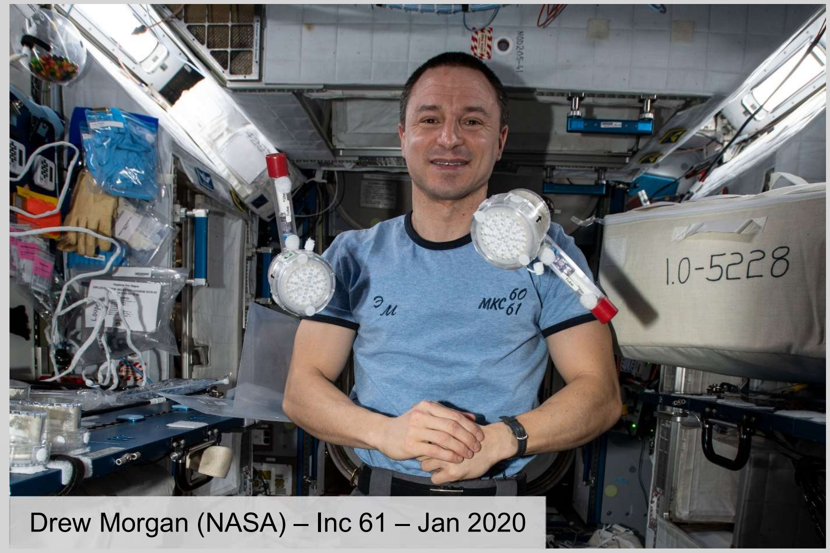
- **Launch**: NG-11, April 17, 2019
- **Onboard Runs**:
  - Run 1: June 2019
  - Run 2: January 2020
  - Run 3: January 2021
  - Run 4: February 2022
  - At least 2 more runs planned to reach 5-year timeline
- **Sample Returns**:
  - Completed: SpX-17, SpX-18, SpX-19, SpX-20, SpX-21, SpX-22, SpX-24
  - Planned: SpX-25, SpX-26, SpX-27, SpX-28, and potentially more
- BN-1 was presented at POIWG #44 in October 2018





Onboard Photos from BioNutrients-1

David Saint-Jacques (CSA) – Inc 59 – June 2019



Drew Morgan (NASA) – Inc 61 – Jan 2020

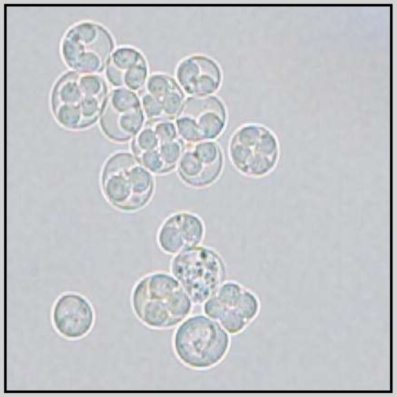
Shannon Walker (NASA) – Inc 64 – Jan 2021

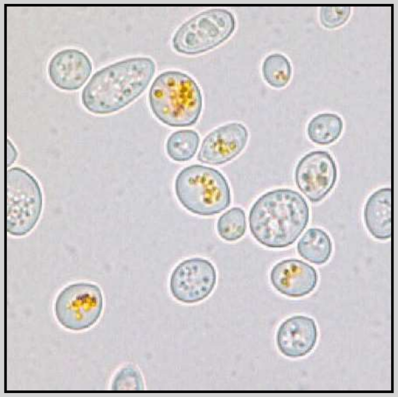
Tom Marshburn (NASA) – Inc 66 – Feb 2022

BioNutrients-2
Hardware Overview

Sample Types - Organisms

Species/Strain	Product	Type	Label Color
Saccharomyces cerevisiae Y55	Zeaxanthin	Yeast	Light Purple
Saccharomyces cerevisiae var. *boulardii*	Beta-carotene	Yeast	Peacock Blue
Kluyveromyces lactis	Follistatin	Yeast	Traffic Grey
Streptococcus thermophilus/ Lactobacillus bulgaricus	Yogurt	Yogurt	Pink
Streptococcus thermophilus	Yogurt with green fluorescent protein (GFP)	Yogurt	Green
Mixed organism culture	Kefir	Kefir	Tan


S. cerevisiae (Zeaxanthin)


S. boulardii (β-carotene)


Yogurt strains


S. thermophilus (GFP)

Hardware Overview

- ISS Production Bag Kits
  - Opened onboard, bags are processed by crew and returned in cold stowage, kit trashed.
  - Each kit contains four Production Bags.
- Earth Production Bag Kit
  - Returned unopened in ambient stowage
  - Each kit contains four Production Bags.
- Support Kit
  - Contains PWD Adapter, Water Bag, and Syringe.
  - Trashed onboard after use
- SABL Interface Board Kit
  - Contains Interface Boards used to attach bags to SABL tray.
- Bitran Bags
  - Secondary containment for bags in cold stowage

Production Bag

Needlefree Swabable Barb Valve

Capped with a non-Luer Thread Cover in ISS Production Bag configuration.
Description: 1/8" barbed fitting, female Luer-Lok, polycarbonate body and barb base, silicone stem

Fluorinated Ethylene Propylene (FEP) Bag

Manufacturer: Instant Systems
Two thicknesses: 3mil used for Yeast, 5mil used for Yogurt/Kefir
Description: 3mil FEP Film or 5mil FEP Film, 8.5" L x 3.25" W

Labels

P/N indicates thickness of bag and whether it was built in earth (-001) or ISS (-002) config.
Color-coded border indicates sample type
S/N range also indicates Sample Type

Support Kit

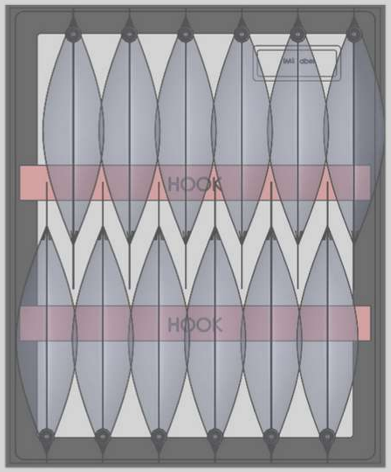
PWD Adapter Assembly, Luer Lock
Provided by: JSC CHeCS Group
Part No.: SEG46121618-301
Description: Interfaces with ISS Potable Water Dispenser

Post-Flight Analysis Bag
Provided by: JSC CHeCS Group
Part No.: SEG46119988-611
Description: 1L water bag that receives water from PWD through PWD Adapter interface

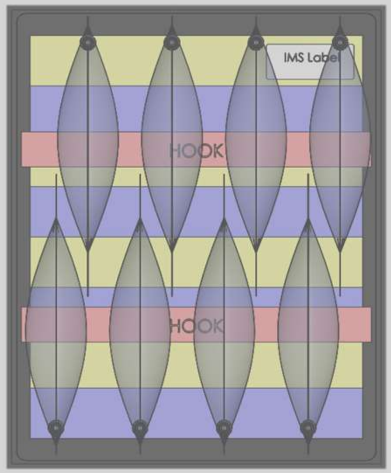
Syringe, Luer-Lok, 50ml Sterile
Manufacturer: Becton Dickinson
Part No.: 309653
Description: 50ml syringe used to draw water from the Post-Flight Analysis Bag

SABL Interface Board

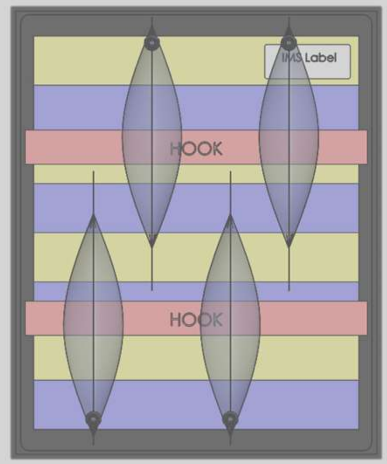
Yeast



Yogurt



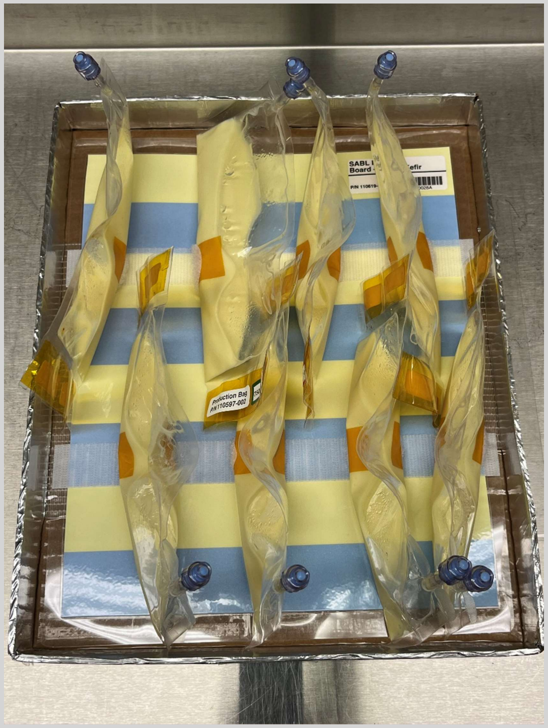
Kefir



pH Indicator - Yogurt

0hr
Initial Hydration

4hr
Mid-point Inspection



24hr
End of Incubation

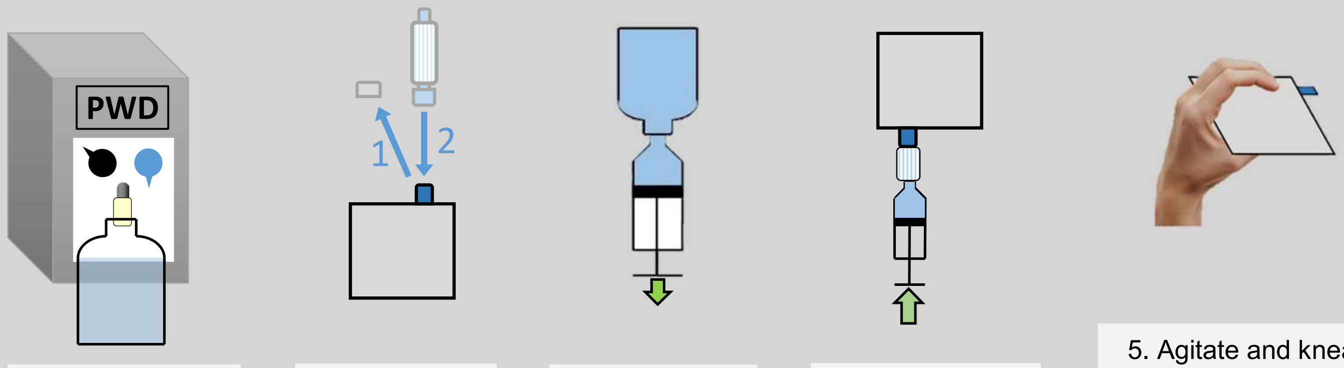
BioNutrients-2 Concept of Operations and Experiment Schedule

Assumptions and Constraints

Assumptions & constraints bound complexity, schedule, and cost, while enabling features needed for quality science:

1. All pre-flight sample and hardware preparation will occur at NASA Ames Research Center.
2. The BioNutrients-2 payload will be launched and returned from the ISS in the SpaceX Dragon spacecraft or comparable vehicle.
3. The BioNutrients-2 payload will be launched to the ISS soft-stowed at ambient temperature.
4. The BioNutrients-2 payload will be stowed at or below 30°C on ISS prior to activation.
5. BioNutrients-2 samples will be maintained at a controlled temperature during the growth phase.
6. BioNutrients-2 samples operated on ISS will be returned at in conditioned cold stowage per requirements.
7. All BioNutrients-2 samples will be early de-stow items.
8. Ground controls will be conducted for all samples in the PI's laboratory.
9. The BioNutrients-2 payload will be delivered to JSC Cargo Mission Contract (CMC) for negotiated late load.

ISS Sample On-Orbit Experiment



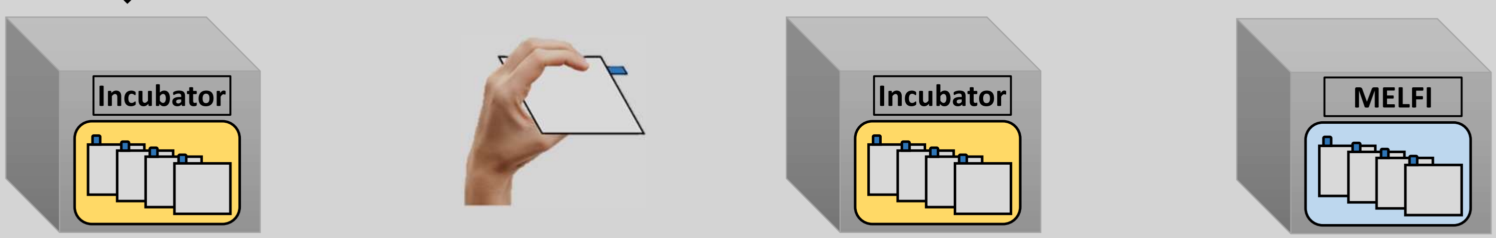
1. Draw water into 1L bag.

2. Remove cap and attach filter

3. Use syringe to draw water from 1L bag.

4. Use syringe to inject water into production bag, then remove filter.

5. Agitate and knead production bag.
Yogurt and Kefir:
After initial agitation, allow to soak for 15min before finishing agitation. Compare to pH guide.



6. Incubate.
Yeast: 30°C
Yogurt: 42°C
Kefir: 24°C

7. Mid-Point
Yeast: Agitate and knead production bag at 6 hours.
Yogurt and Kefir: Inspect and photodocument at 4 hours. Compare to pH guide.

8. Incubate.
Yeast: 30°C (48hrs total)
Yogurt: 42°C (12hrs)→ 4°C
Kefir: 24°C (12hrs) → 4°C
Yogurt and Kefir:
After incubation, compare to pH guide.

9. Place each bag in protective packaging and place in conditioned stowage at -65°C or colder.

Incubation Conditions and Timeline

Set 1

NLT L+45d

YOGURT (incubated together)

- *[4 bags @42°C for 12h; 4°C for 12h] Streptococcus thermophilus/Lactobacillus bulgaricus*
- *[4 bags @42°C for 12h; 4°C for 12h] Streptococcus thermophilus GFP*

NLT L+60d

YEAST (incubated together)

- *[4 bags @30°C for 48h] Saccharomyces cerevisiae* Y55
- *[4 bags @30°C for 48h] Saccharomyces cerevisiae* Boulardii
- *[4 bags @30°C for 48h] Kluyveromyces lactis*

KEFIR

- *[4 bags @24°C for 12h; 4°C for 12h] Kefir-producing mixed organism culture*

Set 2

L+180d [±30d]

YEAST (incubated together)

- *[4 bags @30°C for 48h] Saccharomyces cerevisiae* Y55
- *[4 bags @30°C for 48h] Saccharomyces cerevisiae* Boulardii
- *[4 bags @30°C for 48h] Kluyveromyces lactis*

KEFIR

- *[4 bags @24°C for 12h; 4°C for 12h] Kefir-producing mixed organism culture*

YOGURT (incubated together)

- *[4 bags @42°C for 12h; 4°C for 12h] Streptococcus thermophilus/Lactobacillus bulgaricus*
- *[4 bags @42°C for 12h; 4°C for 12h] Streptococcus thermophilus GFP*

Operations Timeline

Ascent

NG-18 (8/15/2022)
All BioNutrients-2 hardware launched

72 **Earth** Production Bags

12 Y55	12 *boulardii*	12 *lactis*
12 *bulgaricus*	12 GFP	12 Kefir

72 **ISS** Production Bags

12 Y55	12 *boulardii*	12 *lactis*
12 *bulgaricus*	12 GFP	12 Kefir

On-orbit

NLT Launch +45d (9/29/2022)

8 **ISS** Production Bags operated

4 *bulgaricus*	4 GFP

NLT Launch + 60 days (10/14/2022)

16 **ISS** Production Bags operated

4 Y55	4 *boulardii*
4 *lactis*	4 Kefir

Launch + 180d (± 30d) (~Feb 2023)

24 **ISS** Production Bags operated

4 Y55	4 *boulardii*	4 *lactis*
4 *bulgaricus*	4 GFP	4 Kefir

Descent

SpX-26 (12/2022)

24 **Earth** Production Bags returned

4 Y55	4 *boulardii*	4 *lactis*
4 *bulgaricus*	4 GFP	4 Kefir

SpX-26 (12/2022)

24 **ISS** Production Bags returned

4 Y55	4 *boulardii*	4 *lactis*
4 *bulgaricus*	4 GFP	4 Kefir

SpX-27 (2/2023)

24 **Earth** Production Bags returned

4 Y55	4 *boulardii*	4 *lactis*
4 *bulgaricus*	4 GFP	4 Kefir

SpX-27 (2/2023)

24 **ISS** Production Bags returned

4 Y55	4 *boulardii*	4 *lactis*
4 *bulgaricus*	4 GFP	4 Kefir

BN-1 and BN-2 Comparison

BioNutrients-1	BioNutrients-2
Hard Production Pack	Soft Production Bag
Two Organisms (2 yeast)	Six Organisms (3 yeast, 2 yogurt, 1 kefir)
8 Packs per Run	Either 12 (yeast), 8 (yogurt) or 4 (kefir) bags per run
Runs done over 5 years, approximately once per year	Set of 3 Runs done within L+60d, second set of 3 runs done at L+6mo (±1mo)
0.2µm Filter Pre-attached on ground	0.2µm Filter removed from sterile packaging and attached by crew
Samples incubated at 30C for 48hrs	No change for yeast, but yogurt and kefir are incubated at different temperatures and for shorter times
Packs attached directly to bottom of SABL Tray	Bags attached to SABL Interface Board, which attaches to top of SABL Tray

BN-1 and BN-2 Commonalities

BioNutrients-1	BioNutrients-2
Hardware launched at ambient temperature	
Subset of samples returned unopened in ambient storage (Earth Packs/Bags) as "Flown Controls"	
Subset of samples processed onboard, returned in cold stowage (ISS Packs/Bags)	
Payload hardware does not require power, data, or commanding	
Payload incubated in SABL, utilizing fan, door crack, and SABL Short Tray	
Water collected from PWD, injected using syringe	
Requesting two video streams: MWA View (Node 2) + SABL View (LAB/JEM/COL)	
Crew works at MWA with over-the-shoulder video and S/G enabled PD	
Samples are HRL 0, but kit does contain HRL 1 components	

Manifest Overview

Manifest Overview

Ascent: NG-18

Soft Stowage

- ISS Production Bag Kits (x18)
  - Three kits each of the six organisms
  - Each kit contains four Production Bags, for a total of 72 Production Bags, ISS config.
  - Includes 6 Spare Kits (1 of each organism)
- Earth Production Bag Kit (x18)
  - Three kits each of the six organisms
  - Each kit contains four Production Bags, for a total of 72 Production Bags, Earth config.
  - Includes 6 Spare Kits (1 of each organism)
- Support Kit (x11)
  - Contains PWD Adapter, Water Bag, and Syringe. Trashed onboard after use
- SABL Interface Board Kit
  - Contains Interface Boards (one for Yeast, one for Yogurt/Kefir) used to attach bags to SABL tray. Trashed onboard after use.
- Bitran Bags (x80)
  - Secondary containment for bags in cold stowage

Return #1: SpX-26

Soft Stowage

- Earth Production Bag Kit (x6)
  - One kit of each of the six organisms
  - Early retrieval at KSC

Cold Stowage

- ISS Production Bag (x24)
  - 4 bags of each of the six organisms
  - -70°C or colder for Yeast (Qty: 12 bags)
  - -32°C DCB return acceptable for Yogurt/Kefir (Qty: 12) but prefer -70°C for long-term storage.
  - Early retrieval at KSC

Return #2: SpX-27

Identical to Return #1 shown above

Payload Safety Package Overview and Status

Safety Reviews

- Hazard reports were approved at Phase II level with some standard open work.
- One Non-Compliance Report (NCR) in work:
  - NCR addresses temporary lack of fault tolerance regarding containment of Tox 1 materials (oxygen scavenger and desiccant) when kit is cut open
  - The Equivalent Safety designation allows the NCR to be approved by the ISS Safety Review Panel and it will not require approval by the ISS Program

Safety Review	Date
Phase I	4/28/2021
Phase II	10/21/2021
Phase III	June 2022 (planned)


THANK YOU!